CLAIMS

1. A method for generating a peptide internal standard, comprising:
    a) identifying a real or predicted peptide digestion product of a target polypeptide;
    b) determining the amino acid sequence of the peptide;
    c) synthesizing a peptide comprising the amino acid sequence of the peptide digestion product;
    d) labeling the peptide with a mass-altering label;
    e) fragmenting the peptide and identifying a peptide signature diagnostic of the peptide.

2. The method according to claim 1, further comprising selecting a labeled synthesized peptide which co-elutes with an unlabeled peptide consisting of the same amino acid sequence in a chromatographic separation procedure.

3. The method according to claim 1, further comprising fragmenting one or more fragments of the peptide obtained in step, until a peptide signature diagnostic of the peptide is obtained.

4. The method according to claim 1, wherein fragmenting is performed by multistage mass spectrometry.

5. The method according to claim 1, wherein the label is a stable isotope.

6. A method for determining the presence and/or quantity of a target polypeptide in at least one mixture of different polypeptides, comprising:
    a) providing a mixture of different polypeptides;
    b) adding a known quantity of a peptide internal standard labeled with a mass-altering label, thereby generating a spiked mixture, wherein the labeled peptide internal standard comprises a subsequence of the target polypeptide and wherein the labeled peptide internal standard possesses a known peptide fragment signature diagnostic of the presence of the peptide;

c) treating the spiked mixture with a protease activity to generate a plurality of peptides including the labeled peptide internal standard and peptides corresponding to the target polypeptide;

d) fragmenting the labeled peptide internal standard and any target peptide present in the spiked mixture comprising the same amino acid sequence as the labeled peptide internal standard;

e) determining the ratio of labeled fragments to unlabeled fragments; and

f) calculating from the ratio and the known quantity of the labeled internal standard, the quantity of the target polypeptide in the mixture.

7. The method of claim 6, wherein the fragmenting is performed by multistage mass spectrometry.

8. The method of claim 6, further comprising separating peptides obtained in step (c) using a chromatography step.

9. The method according to claim 8, wherein the chromatography step comprises performing HPLC.

10. The method according to claim 9, wherein the labeled peptide internal standard and target peptide comprising the same amino acid sequences as the labeled peptide internal standard are co-eluted during separation.

11. The method according to claim 6, wherein the mixture of different polypeptides is selected from the group consisting of: a crude fermenter solution, a cell-free culture fluid, a cell or tissue extract, blood sample, a plasma sample, a lymph sample, a cell or tissue lysate; a mixture comprising at least about 100 different polypeptides; a mixture comprising substantially the entire complement of proteins in a cell or tissue.

12. The method according to claim 6, wherein the peptide internal standard is labeled using a stable isotope.

13. The method according to claim 6, wherein the labeled peptide internal standard is produced according to the method of claim 1.

14. The method according to claim 6, wherein the presence and/or quantity of target polypeptide is diagnostic of a cell state.

15. The method according to claim 14, wherein the cell state is representative of an abnormal physiological response.

16. The method according to claim 15, wherein the abnormal physiological response is diagnostic of a disease.

17. The method according to claim 14, wherein the cell state is a state of differentiation.

18. The method according to claim 6, further comprising determining the presence and/or quantity of target peptides in at least two mixtures.

19. The method according to claim 18, wherein one mixture is from a cell having a first cell state and the second mixture is from a cell having a second cell state.

20. The method according to claim 20, wherein the first cell is a normal cell and the second cell is from a patient with a disease.

21. The method according to claim 18, wherein the determining is done in parallel.

22. The method according to claim 18, wherein the two mixtures are the same and the labeled peptide internal standard is provided in different known amounts in each mixture.

23. The method according to claim 1, wherein the label is associated with an amino acid residue which is known or predicted to be modified in the target polypeptide.

24. The method according to claim 23, wherein the label is attached to a modified amino acid residue.

25. The method according to claim 18, wherein the labeled peptide internal standard in each mixture comprises the same peptide but different labels.

26. A labeled peptide internal standard comprising a peptide comprising a stable isotope label attached to peptide comprising a modified amino acid residue; wherein the peptide is identical in amino acid sequence to an amino acid subsequence of a target polypeptide and wherein the modified amino acid residue corresponds to a known or putative modified amino acid residue in the target polypeptide.

27. The peptide internal standard according to claim 26, wherein the modified amino acid residue is a phosphorylated residue, a glycosylated residue, a ubiquitinated residue, a ribosylated residue, and acetylated residue or a farnesylated residue.

28. A panel of labeled peptide internal standards comprising corresponding to different amino acid subsequences of a single polypeptide.

29. The panel according to claim 28, further comprising a peptide internal standard comprising a modified amino acid residue.

30. A panel of labeled peptide internal standards comprising peptides corresponding to different proteins in a molecular pathway, wherein each peptide comprises a fragmentation signature diagnostic of a protein in the molecular pathway.

31. The panel according to claim 30, wherein the molecular pathway is selected from the group consisting of a JAK pathway, a MAPK pathway, a cell cycle pathway, and a G-Protein Coupled Receptor Pathway.

32. A panel of labeled peptide internal standards comprising a plurality of peptides corresponding to pro-inflammatory cytokines, anti-inflammatory cytokines, and mixtures thereof, wherein each peptide comprises a fragmentation signature diagnostic of a pro- or anti-inflammatory cytokine.

33. A panel of labeled peptide internal standards comprising at least a first peptide internal standard with a fragmentation signature diagnostic of a first disease and at least a second peptide internal standard with a

fragmentation signature diagnostic of a second disease, wherein the first and second diseases are different.

34. The panel according to claim 33, wherein the panel is provided mixed in a single container.

35. The panel according to claim 33, wherein the first and second diseases are different neurodegenerative diseases.

36. The panel according to claim 33, wherein the peptide internal standards correspond to different cancer specific antigens.

37. The panel according to claim 33, wherein the first and second diseases are different respiratory diseases.

38. The panel according to claim 33, wherein the first and second diseases are different autoimmune diseases.

39. The panel according to claim 33, wherein the first and second diseases are different infectious diseases.

40. The panel according to claim 30, further comprising a peptide internal standard comprising a modified amino acid residue.

41. A kit comprising a labeled peptide internal standard comprising a peptide identical in sequence to an amino acid subsequence in a target polypeptide wherein the peptide is labeled with a stable isotope and wherein the kit further comprises software for analyzing mass spectra of peptide fragments.

42. A kit comprising a labeled peptide internal standard comprising a peptide identical in sequence to an amino acid subsequence in a target polypeptide wherein the peptide is labeled with a stable isotope and wherein the kit further comprises a means for providing access to a computer memory comprising data files storing information relating to the diagnostic

fragmentation signatures of one or more peptide internal standards in the kit.

43. The kit according to claim 35, wherein access is provided by including a computer readable program product comprising the memory.

44. The kit according to claim 35, wherein access is provided in the form of a URL and/or password for accessing an internet site for connecting a user to the memory.

45. The kit according to claim 35, wherein the kit comprises data, in electronic or written form, relating to amounts of one or more target proteins characteristic of one or more different cell states.

46. The kit according to claim 35, wherein the kit further comprises expression analysis software on computer readable medium which is capable of being encoded in a memory of a computer having a processor; and wherein the software is further capable of causing the processor to perform a method comprising the steps of:

(a) determining a test cell state profile from peptide fragmentation patterns in a test sample comprising a cell with an unknown cell state or a cell state being verified;

(b) receiving a diagnostic profile characteristic of a known cell state; and

(c) comparing the test cell state profile with the diagnostic profile.